Project Report – FY2006

Project Title: Technologies for Detecting Enteroviruses, and Multiple Drug Resistant *E. coli***.**

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Funding Amount and Period: 0 K, June 2006-2007. No funding was received for 2006. This includes ongoing OHH research

Background and Rationale:

Freshwater runoff contaminated with human or animal wastes from septic systems, inadequately disinfected municipal treatment plant outfalls, and animal facilities is the primary source of infectious pathogenic organisms in estuaries and coastal waters. Exposure to these infectious agents can occur through direct contact with skin lesions or inadvertent consumption of contaminated water during recreational activities, particularly at recreational beaches. Infectious doses of these organisms can often be quite low, so methods designed to detect these pathogens and for use in tracking the initial sources of the contamination must be quite sensitive. Molecular techniques offer a rapid, cost-effective tool for detecting low concentrations pathogenic bacteria, parasites and viruses in environmental samples. This research project concentrated 1) on to developing an accurate quantitative RT- assay for enterovirususes which cause millions of cases of gastrointestinal illness each year and are of particular concern at in recreational areas and 2) on evaluating genetic variation in antibiotic plasmids can be used as a more reliable means to track sources of human and animal fecal contamination into estuaries and coastal regions than currently available. The first assay is specifically designed for monitoring recreational and drinking water supplies so that closures can be imposed if viral loads high enough to cause infection are detected. The logic behind the second assay is that antibiotic resistant plasmids are both genetically variable, and common enough due to the frequent administration of antibiotics to humans and animals, to be used for source tracking. Successful development of these assays requires extensive development of internal controls to assess inhibition, contamination, and assay efficiency. It also requires a reliable method for quantitatively recovering the target DNAs or RNAs from the pathogens in an environmental sample which are the basis for the molecular assays. To date most of the work has concentrated on the enteroviral assay.

Geographic Scope: This project is being conducted in California as well as North and South Carolina. Potential partners include the public health department in each of these states.

Objectives:

- Develop accurate and sensitive quantitative RT-PCR methods for detecting enteroviruses which cause millions of cases of gastroenteritis each year
- Determine efficacy of using antibiotic resistant plasmids to track sources of fecal contamination
- Determine optimal extraction techniques for processing environmental samples
- Develop standardized assay controls to monitor for inter assay efficiency, the presence of inhibitors and the possibility of cross-contamination

Accomplishments/Progress to Date:

- A standardized assay has been developed for enteroviruses
- The assay has been successfully tested in samples from California and North Carolina
- A description of the assay has been accepted with revisions in Applied and Environmental Microbiology
- The assay will be used to test for enteroviral contamination in South Carolina waters
- The optimal extraction technique and the maximum volume of filtered water that can be successfully processed one time have been determined
- Novel internal controls to monitor for assay sensitivity and inhibition have been developed
- The assay has been published in Applied and Environmental Biology

Anticipated Products and Major Findings:

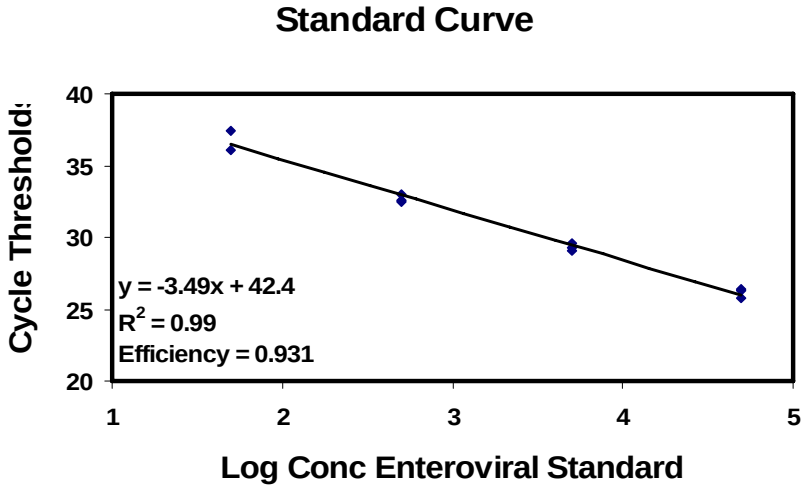
Products

- An enteroviral assay that can be used by state public health officials and is now being used to monitor estuarine and coastal waters for viral loads of public health concern
- Development of internal inhibition control that will prove useful in monitoring many other types of environmental pathogens as well

Intended audience

- Public Health officials
- Resource managers

Graphics: The graphic below shows the large dynamic range of the quantitative RT- PCR assay for enteroviruses



Issues:

- The enteroviral assay required additional testing in different environments to ensure that the current extraction techniques are broadly applicable, and would benefit from development of an extraction control
- Extensive sequencing of target genes in antibiotic plasmids needs to be undertaken as the next step in that project

- Once suitable target plasmid sequences are identified, extraction techniques based on what was learned from the enterovirus extraction procedures will be used to screen and amplify the same regions from field samples to determine if the assays will have sufficient sensitivity

Microcystins in the Great Lakes (Done in Collaboration with Juli Dyble and Gary Fahnenstiel, GLERL)

Background and Rationale:

Bloom-forming cyanobacterial are found in lakes and reservoirs worldwide and can produce a range of toxins, including hepatotoxic microcystins and cylindrospermopsin as well as neurotoxic anatoxins. The most common cyanotoxins found in the Great Lakes are microcystins, cyclic heptapeptide compounds that are detrimental to human, animal and ecosystem health. The primary source of microcystins is the colony-forming cyanobacteria *Microcystis*, and in particular *Microcystis aeruginosa*, which typically dominates the cyanobacterial bloom community in the Great Lakes. Preliminary studies have documented the presence of microcystins in the Great Lakes, at times exceeding the recommended limit of 1 μg L^{-1} of microcystin established by the World Health Organization for drinking water supplies. Due to the dependence on these waters as a drinking water and recreational resource, an increase in large *Microcystis* blooms in recent years has caused considerable concern. The ability to accurately measure the distribution and concentration of microcystin in the Great Lakes, including the various microcystin congeners, is therefore essential to protect human and ecosystem health in this region. The purpose of this study was to systematically map intracellular and extracellular microcystin concentrations in eutrophic portions of the Great Lakes during a summer period when *Microcystis* sp. were abundant. A complication in assessing this toxicity is that the toxin genes are carried on a genetic cassette with some strains having the capacity to produce toxins and other not. Morphologically these toxic and non-toxic strains are identical. These studies therefore also investigated the relationship between microcystin concentrations and both *Microcystis* colony abundance and the frequency of toxic genotypes in the *Microcystis* population during the cruises.

Geographic Scope: Great Lakes and surrounding states

Objectives:

- Estimate the concentrations of microcystins found in the drinking water supply for approximately 5 million people in the US and Canada.
- Investigate the genetic and environmental factors regulating bloom formation and toxicity.

Accomplishments/Progress to Date:

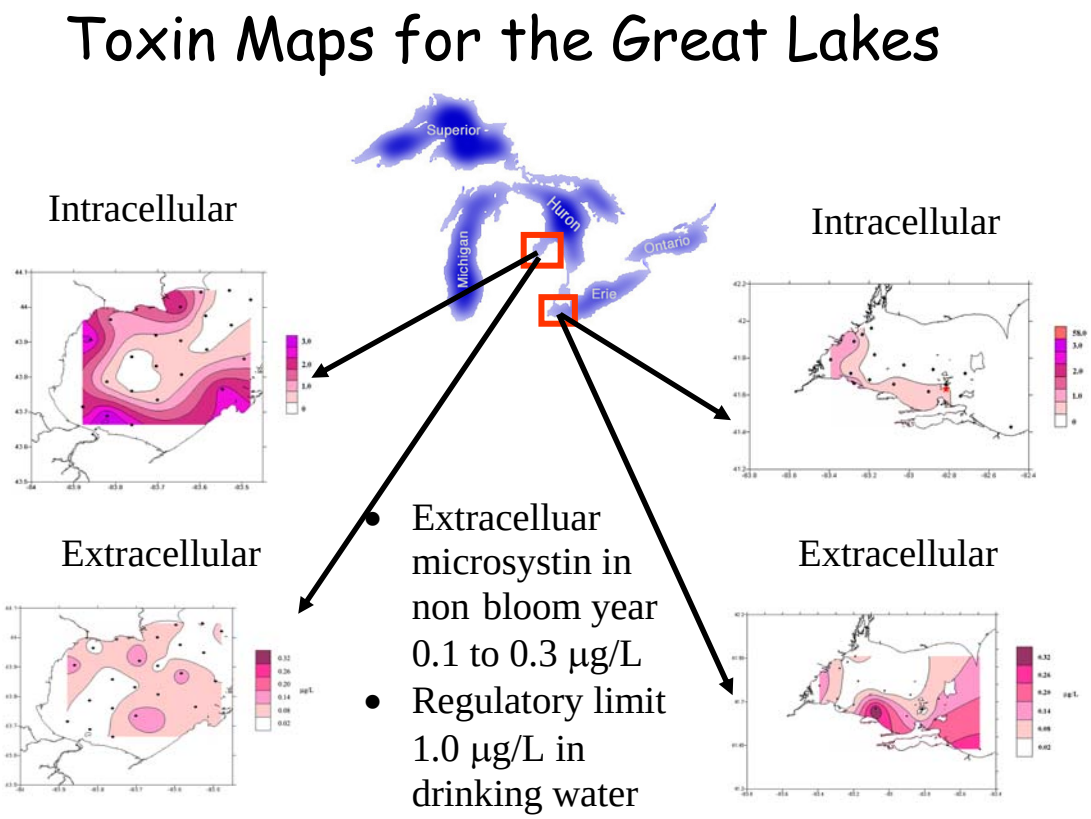
- Sampled Saginaw Bay, Lake Huron and western Lake Erie during cruises in 2004, 2005 and 2006
- Measured chlorophyll *a*, HPLC (phytoplankton photopigments), nutrients, growth rates, intra- and extra-cellular microcystin toxin concentrations and cell counts.

- Performed molecular assays to determine proportion of toxic genotypes in a bloom community.
- Begun to examine environmental regulators of toxicity
- Produced maps of toxin levels that have been made available to managers

Anticipated Products and Major Findings:

- During bloom years microcystin levels could be above the 1 μg l^{-1} level recommended in finished water as safe by the World Health Organization.
- The toxicity of the bloom will depend on the proportion of toxic genotypes in the population

Graphics:



Issues:

- Using remote sensing to monitor for potential threat to public health is complicated by the fact that the toxicity of a bloom is influenced by the genetic make-up (proportion of toxic and non-toxic cells) of the population as by overall biomass.
- Occasionally other toxin producing cyanobacteria will constitute a significant portion of the population and so toxins other than microcystins will need to be monitored.
- Current method of closing on the basis of cell counts does not correlate well with toxicity and needs to be revised.